# BIOGENIC MONOAMINES CHANGE IN ISCHEMIC RAT CEREBRAL CORTEX

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## ABSTRACT

Adult, male Sprague-Dawley rats subjected to 15 minutes of temporal unilateral common carotid artery occlusion and up to 6 hours of re-circulation was employed. The cortical concentration of noradrenalin(NA), dopamine(DA), serotonin(5-HT)and their metabolites, vainyllmandelic acid(VMA), homovanillic acid(HVA), 5-hydroindoleacetic acid(5-HIAA),were determined by High Performance Liquid Chromatography with electrochemical detection. After 6 hours of recirculation, diminution of NA, DA and 5-HT, accumulation of VMA, HVA and 5-HIAA were noted in ischemic cortex. This findings strongly suggested that a postischemia increase release and turnover of NA, DA and 5-HT, which may explain delayed postischemic hypoperfusion which was caused by postischemic vasoconstrictions.

**Key Word :** noradrenalin, vanillmandelic acid, dopamine, homovanillic acid, serotonin, 5-hydroindoleacetic acid

## INTRODUCTION

Patient with stroke often deteriorate while being observed hours or even days after the inital ictus. Although immediate treatment of stroke is probably impractical, the delayed deteriorate would be prevented if the responsible pathologic processes were understood. During the past decade, there has been a surge of interest in the possible relationship between brain monoamine and ischemic brain insults. Release of vasoactive amines, formation of vasoactive product of arachidonic acid metabolism, and disturbance of calcium ion hemostasis in cerebrovascular smooth muscle may attribute to postischemic vasoconstriction[1]. Monoamine transmitters may play an important role in postischemic cerebral dysfunction. Monoamine may regulate cerebral reperfusion after ischemic insult and influence the recovery of brain ischemia.

The purpose of this investigation was to examine change of noradrenalin, dopamine, serotonin and their metabolites VMA, HVA and 5-HIAA in transient ischemic cerebral cortex. Thus, a major objective of our research is to quantify these agents and their metabolites then to compare their differences between the control cortex and ischemic cortex using a rat model. The results obtained in the present study clearly

show that significant differences occur in control and ischemic cortex tissues.

## MATERIALS AND METHODS

Adult, fifteen male Sprague-Dawley rat weight 200-250 gm were used and caged( ( three per cage)at constant temperature about 27 ℃ in simulated day and night condition for 3 weeks prior to study and free access to drinking water and Lab chow. The rats were anesthesized with ketamine hydrochloride(ketalar 100mg/Kg intraperitoneally). Left common carotid artery were exposed through ventral midline incision and arteries, isolated from related nervous and vessels, were clipped with Sugita aneurysm clip for 15 minutes, then was released. The animals were killed by decapitation 6 hours after transient ischemia. The heads were severed, cooled in an ice bath, and the brains were removed(Lt ischemic brain) . In control group, three rats subjected to sham operation without clipping the left common carotid artery, left cortex also was removed 6 hours later. The concentration of noradrenalin, dopamine, serotonin and their metabolites VMA, HVA and 5-HIAA in both cortex were analyzed by High Performance Liquid Chromatography with electrochemical detection(HPLC, Waters). The mobile phase is a water-methanol mixture, and the column is a E.Merk RP-18 endcapped column.

All values obtained from this experiment are expressed as mean ± SEM. The significance between control and experimental data determined by student's-t-test.

## RESULTS

Control cortex from male had detectable NA, DA, 5-HT and their metabolites VMA, HVA, 5-HIA. The concentration(mean ± SEM) of NA, DA, 5-HT and their metabolites VMA, HVA, 5-HIAA in control cortex were 684 ± 32, 384 ± 64,268 ± 20, and 286 ± 16,746 ± 54,574 ± 26 ng per gram(n=3)are given in Tab. 1 and Fig. 1, respectively. The ischemic cortex from male had detectable NA, DA, 5-HT and their metabolites VMA, HVA and 5-HIAA. The concentration(mean ± SEM)of NA, DA, 5-HT and their metabolites VMA, HVA, 5-HIAA in ischemic cortex were 326 ± 18,214 ± 26,108 ± 18 and 502 ± 18, 1684 ± 124, 964 ± 16, ng per gram(n=15)are given in Tab. 1 and Fig. 2, respectively. The difference are statistically significant between control and ischemic cortex.

Table 1. The concentration of biogenic monoamines and their metabolites in control and ischemic rat cortex

| Tissue | NA | VHA | DA | HVA | 5-HT | 5-HIAA |
|---|---|---|---|---|---|---|
| Cortex | 684±32 | 288±16 | 384±64 | 746±54 | 268±20 | 574±26 |
| Ischemic cortex | 326±18a | 502±18b | 214±26c | 1684±24d | 108±18e | 964±16f |

aDifferent from cortex $p<0.01$ by two-tailed Student's t-test
bDifferent from cortex $p<0.01$ by two-tailed Student's t-test
cdifferent from cortex $p<0.05$ by two-tailed Student's t-test
dDifferent from cortex $p<0.001$ by two-tailed Student's t-test
eDifferent from cortex $p<0.01$ by two-tailed Student's t-test
fDifferent from cortex $p<0.05$ by two-tailed Student's t-test
values are means ± SE of five separate preparation
NA=Noadrenalin
VHA=Vainyllmandelic Acid
DA=Dopamine
HVA=Homovanillic Acid
5-HT=Serotonin
5-HIAA=5-Hydroindoleacetic Acid

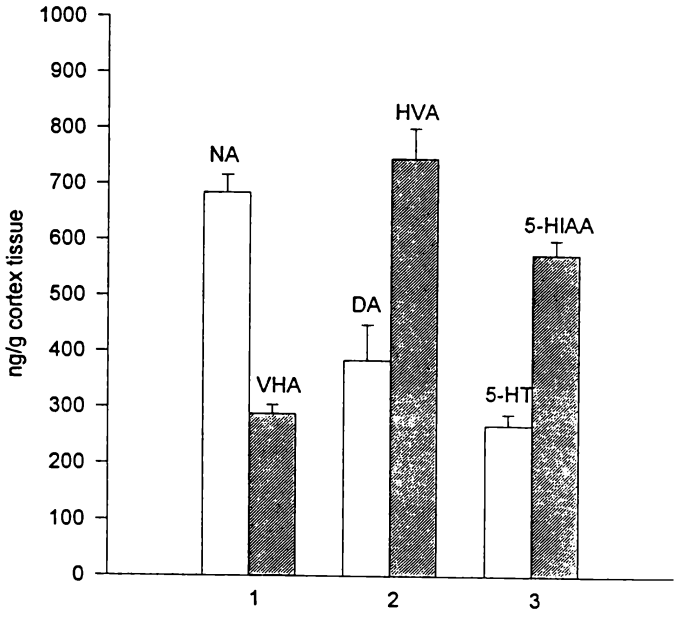


Fig 1 : The concentration of biogenic monoamines and their metabolites in control rat cortex.

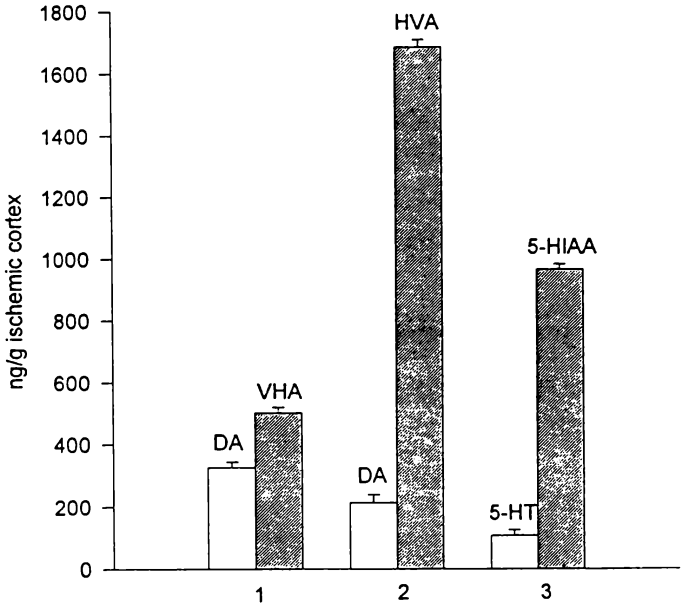


Fig 2 : The concentration of biogenic monoamines and their metabolites in ischemic rat cortex.

## DISCUSSION

In the recirculation period following cerebral ischemia, an initial reactive hyperemia is succeeded by a secondary decrease in cerebral blood flow(CBF), called delayed postischemic hypoperfusion[2-7]. Cerebral recovery may be hindered by poor perfusion rather than by immediate death of hypoxic nerve cells. The hyperemia may be explained by vasodilation due tissue acidosis from lactic acid and carbon dioxide, or by a slight increase in extracellular potassium[8]. The cause of impaired cerebral microcirculation following ischemia has been attributed to many factors. Ames and co-workers concluded that the " no-reflow phenomenon " was due to increase blood viscosity and endothelial and perivascular glial cell swelling[9]. Ito concluded that arterial spasm was responsible glial cell swelling[10]. Fluresecent histochemical study have demonstrated that biogenic monoamines (NA, DA, 5-HT) in axon terminals of the contex, to which they project from brain stem neurons. Some investigators have been assigned a regulatory role in cerebral blood flow (CBF)to biogenic monoamine, and this concept has been supported by observed effects of NA, DA, 5-HT on pial vessel diameter[11]. On the basis of the vasoactive properties of these monoamines, they has been speculated that the increased turnover of these amines could lead to deleterious vasoconstriction, which might cause additional cellular damage[12]. This study showed that deprivation of blood to the brain led to reduction of NA, DA and 5-HT concentration in the cerebral cortex. This reduction is due to the increased release of these monoamines, since the rates of its synthesis and degradation are depressed after vascular occlusion. Wurtman and Zervas have postulated that an inappropriate loss of monomine neurotransmitter may exacerate the pathophysiologic changes cause by initial ischemia[13]. Reduction of biogenic monoamines concentration in cerebral cortex appeared at the time when adenosine triphosphate levels already were reduced for a long period. Unlike many other cell types, neuron in vivo appear incapable of using any other substance than glucose as their main energy source. Under normal circumstances, adenosine triphosphate, the energy source for the maintenance of the electrochemical gradients, is efficiently produced through aerobic glycolysis and oxidative phosphorylation. Cerebral ischemia which is characyerized by deprivation of glucose and oxygen supply to the brain, inevitable led to ATP depletion. Therefore, it appears that release of biogenic monoamines in the brain is most probably related to a failure of the neuronal energy stores and the membrane transport following vascular occlusion[14]. Zeras, Hori and Wurtman have suggested that a fall in biogenic monoamines reflects release of thses transmitter into extracellular fluid where it may have the effect of limiting capillary perfusion by vasoconstriction small

vessels[15]. The post-ischemic disruption of flow and metabolism, therefore, creates a potential for further brain damage. This secondary change might be prevented by appropriate therapeutic intervention because it occurs at time when patient are often under medical supervision.

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## REFERRENCE

1.Levy DE, Uitert RLV, Pike CL : Delayed postischemic hypoperfusion. A potentially damaging consequence of stroke. Neurology 1979;29:1245-1252.

2.Hossmann KA, Lechtape-Gruter H, Hossmann V : The role of the cerebral blood flow for the recovery of the brain after prolonged dischemia. Z Neurol 1973; 204:281-399.

3.Synder JV, Nemoto EM, Carrol RG : Global ischemia in dogs : Intracranial pressure, brain flow and metabolism. Stroke 1975; 6:21-27.

4.Nordstrom CH, Rehncrona S : Postischemic cerebral blood flow and oxygen utilization rate in rat anesthesized with nitrous oxide or phenobarbital. Acta Physiol Scand 1977;101: 230-240.

5.Pulsinelli WA, Levy DE, Duffy TE : Regional cerebral blood flow and glucose metabolism following transient forebrain ischemia. Ann Neurol 1982;11:498-509.

6.Kagostrom E, Smith ML, Siesjo BK : Local cerebral blood flow in the revovery period following complete ischemia in the rat. J Cere Blood Flow Metab 1983;3:170-182.

7.Chikashi M : Monoamine metabolism after transient global ischemia. Neuro Med Chir (Tokyo)1989;29:1093-1100.

8.Cameron IR, Segal MB : The effect on pial arteriole diameter of local changes in potassium concentration. Euro Neurol 1971;6:100-106.

9.Ames A, Wright RL, Kowada M : Cerebral ischemia Ⅱ , The no-reflow phenomenon. Amer J Path 1968;52:437-447.

10.Ito U, Spatz M, Walker JT Jr, Klatzo I : Experimental cerebral ischemia in Monogolian gerbils. 1.Light microscopic observations. Acta Neuropathologica 1975;98:388-93.

11.Welch KMA, Chabi E, Buckkingham J : Catecholamine and 5-hydroxytrypamine level in ischemia brain. Stroke 1977;8:584-690.

12.Melamed E, Moskowitz MA, Wurtman RJ : Involvement of monoamines in the pathogenesis of cerebral ischemia. Amsterdam, Excerta Medica 1980;173-182.

13.Wurtman RJ, Zervas NT : Monoamine neurotransmitters and the pathophysiology of stroke and central nervous system. J Neurosurgery 1974;4034-6.

14.Mrsulija BB, Mrsulija BJ, Spata M : Brain serotonin after experimental vascular occlusion. Neurology 1976;26:785-787.

15.Zervas NT, Hori H, Wurtman RJ : Reduction in brain dopamine following experimental cerebral ischemia. Nature 1974; 247:283-284.

# 腦缺血對大鼠大腦皮質 Biogenic Monoamines 濃度改變之研究

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雄性大白鼠左側頸動脈以血管夾夾住 15 分鐘後釋放，於再循環六小時後斷頸取出其大腦，以 High Performance Liquid Chromatography with Electrochemical Detection 測量左、右大腦皮質 Noradrenalin(NA) 、 Dopamine(DA) 、 Serotonin(5-HT) ，及其代謝物 Vainyllmandelic acid(VMA) 、 Homovanillic acid(HVA) 、 5-Hydroindoleacetic acid (5-HIAA) 的濃度。對照組僅做頸切開，暴露左頸總動脈，其它與實驗組相同。結果左側缺血大腦皮質 NA 、 DA 、 5-HT 的濃度較對照組減少，而其代謝物 VMA 、 HVA 、 5-HIAA 的濃度較對照組增加。

由此實驗結果，可認爲腦缺血缺氧後 Biogenic Monoamines 釋放及轉變的增加是造成腦缺血後血管收縮的原因。

**關鍵詞：** Noradrenalin 、 Dopamine 、 Serotonin 、 Vainyllmandelic acid 、 Homovanillic acid 、 5-Hydroindoleacetic acid